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The Crucial Role of Environmental Coronas in Determining the Biological Effects of Engineered Nanomaterials

Lining Xu, Ming Xu,* Ruixia Wang, Yongguang Yin, Iseult Lynch, and Sijin Liu*

In aquatic environments, a large number of ecological macromolecules (e.g., natural organic matter (NOM), extracellular polymeric substances (EPS), and proteins) can adsorb onto the surface of engineered nanomaterials (ENMs) to form a unique environmental corona. The presence of environmental corona as an eco–nano interface can significantly alter the bioavailability, biocompatibility, and toxicity of pristine ENMs to aquatic organisms. However, as an emerging field, research on the impact of the environmental corona on the fate and behavior of ENMs in aquatic environments is still in its infancy. To promote a deeper understanding of its importance in driving or moderating ENM toxicity, this study systemically recapitulates the literature of representative types of macromolecules that are adsorbed onto ENMs; these constitute the environmental corona, including NOM, EPS, proteins, and surfactants. Next, the ecotoxicological effects of environmental corona-coated ENMs on representative aquatic organisms at different trophic levels are discussed in comparison to pristine ENMs, based on the reported studies. According to this analysis, molecular mechanisms triggered by pristine and environmental corona-coated ENMs are compared, including membrane adhesion, membrane damage, cellular internalization, oxidative stress, immunotoxicity, genotoxicity, and reproductive toxicity. Finally, current knowledge gaps and challenges in this field are discussed from the ecotoxicology perspective.

result in the unpredictable release of nanoparticles (NPs) into aquatic environments such as lakes, rivers, oceans, and wetlands; this has raised significant concern on the impact of ENMs on environmental health and safety.^[9,10] Various ENMs (e.g., metallic, metal oxide, carbonaceous, and polymeric NPs) trigger different types of emerging risks for aquatic ecology. For instance, it has been reported that the concentration of TiO₂ NPs in the Old Danube Recreational Lake is seasonally dependent, based on the significant release of these NPs from sunscreen into surface waters during bathing season.^[11] Emissions of TiO₂ NPs from paints and Ag NPs from textiles into the aquatic environment have also been observed.^[12,13] Increasing ecological risks and potential food chain risks for humans from these NPs in aquatic environments have attracted considerable research attention.^[14–16]

When ENMs come into contact with the body fluids of mammals (e.g., blood, lymph, alveolar fluids) through ingestion, inhalation, or other means, a myriad of biomolecules is adsorbed onto their surface, forming a “biocorona.” This endows

ENMs with a new biological identity,^[17,18] which is distinct from but influenced by the intrinsic physicochemical properties of the ENMs prepared in the laboratory or manufacturing plant. To illustrate this phenomenon, the concept of the “protein corona” has been proposed to depict the protein layers that adsorb onto ENMs in biological milieu approximately one decade ago.^[19] Since this time, there has been an intense focus on elucidating the nature and role of the protein corona in nanomedicine

1. Introduction

Engineered nanomaterials (ENMs) show great potential as raw materials or as vital additives in water and wastewater treatment,^[1–5] sterilization and disinfection, and personal-care products.^[4,6–8] This is because of their high surface area-to-volume ratio, abundant adsorption binding sites, and excellent catalytic properties. As a consequence, human activities may

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and nano-safety. This has greatly promoted the knowledge and application of ENMs due to multidisciplinary efforts from nanoscience, chemistry, physics, biology, and toxicology. It has been found that the protein corona formed in biological fluid is associated with the bio-nano interactions of ENMs. For example, bioavailability, metabolism, immune response, and toxic endpoints, are attributable to the manifold physiological functions of protein molecules.^[20,21] ENMs can also be coated by other biomacromolecules, such as lipids^[22,23] and other metabolites.^[24,25] Thus, the continuous extension of the bio-corona will most certainly advance health-related disciplines, such as cancer therapy, disease diagnosis, and occupational protection.^[26]

Although investigations regarding the fate and effects of ENMs in aquatic environments commenced at the beginning of this century, the role of ecological macromolecules on the toxicological risks of ENMs has received limited attention in most reported studies.^[27–29] In recent years, it has been found that in aquatic environments, macromolecules may adsorb onto the surface of ENMs forming an environmental corona;^[24,30–32] this corona is also known as the ecological corona or macromolecular corona.^[30,32,33] In this review, the term environmental corona was used to represent the formation of macromolecular and small molecule (metabolite) corona on ENMs in aquatic environments. Although key literature on this topic has systematically reviewed the environmental corona from various perspectives, there continue to be many knowledge gaps that hamper a deep understanding of its impact on the fate and ecological risks of ENMs.^[9] In particular, the laboratory conditions applied in most reported studies are quite different from the conditions in aquatic ecosystems.^[34] Unlike physiological conditions, ecological macromolecules (e.g., natural organic matter (NOM), extracellular polymeric substances (EPS), proteins, metabolites, and surfactants), are much more heterogeneous and dynamic in reality, generating distinct environmental identities. According to previous studies, the presence of ecological macromolecules alters ENMs in three key aspects; i) physicochemical properties, ii) environmental fate, and iii) biological interactions.^[9,35–38] To date, of these three aspects, there is little known regarding (iii), and knowledge on biological interactions is still in its infancy. More importantly, an environmental corona may alter the bioavailability of ENMs to multifarious aquatic organisms (e.g., bacteria, algae, crustaceans, and fish), causing many unknown ecological risks and ultimately threatening human health through food chain transport.^[28,39,40] As such, it is necessary to update recent progress and findings in this field so that a risk assessment regarding the potential impacts of ENMs on aquatic ecosystems and human health is based on the best available information.

This review aims to illustrate the representative environmental corona (i.e., NOM, EPS, proteins, and surfactants) of ENMs in aquatic environments, and synthesize recent findings according to the chemical characteristics of the environmental corona for ENMs. In particular, the impact of acquired environmental corona on biological interactions and toxicological mechanisms between ENMs and aquatic organisms at different trophic levels (bacteria, algae, *Daphnia magna* (*D. magna*), bivalves, and fish) will be highlighted. This includes membrane adhesion, membrane damage, cellular internalization, oxidative stress, immunotoxicity, genotoxicity, and reproductive toxicity. Finally, based

on the ecotoxicological perspective, current knowledge gaps and future challenges in this field will be discussed, including the i) characterization of environmental coronas in natural water systems; ii) correlation between corona formations and physicochemical properties of NPs; iii) assessment of the altered ecotoxicity of ENMs by environmental coronas via a battery of aquatic biota; iv) evolution of coronas in abiotic and biotic systems; v) underlying toxicological mechanisms and health risks of the environmental corona-coated ENMs; and vi) latent synergistic or antagonistic effects of ENMs on pollutants (e.g., metal ions) mediated by environmental corona.

2. Environmental Corona Formation on ENMs in Aquatic Environments

2.1. Active Interface between ENMs and Ecological Macromolecules

Metallic (e.g., Ag, Fe, and Au), metal oxide (e.g., TiO₂, ZnO, and CuO), metal sulfide (e.g., Ag₂S, FeS), carbonaceous (e.g., carbon nanotubes, graphene oxide), and polymeric (e.g., polystyrene) NPs have been studied extensively in aquatic ecosystems.^[35,36,41,42] The characteristics of these ENMs vary considerably in terms of size, shape, ligand, charge, rigidity, and roughness. These characteristics determine their overall surface properties. The high surface free energy and distinctive surface properties of ENMs mean that once they are released into natural water systems, ecological macromolecules (e.g., NOM, EPS, proteins), and small molecules (e.g., metabolites) spontaneously adsorb onto their surface, forming the architecture of an eco-nano interface known as the environmental corona (**Figure 1**).^[30–32] At the interface, a complicated interplay of chemical interactions will occur between eco-molecules and ENMs, including covalent bond binding, electrostatic forces, van der Waals forces, and hydrophobic and hydrogen bond interactions. This occurs initially with the most abundant molecules, gradually reorganizing to the highest affinity environmental molecules.^[37] Once the adsorption equilibrium has occurred, a single or multi-layer of eco-molecules will ultimately form on ENMs, generating a new identity for the ENM. As reported previously, the protein corona is composed of long-lived and loosely-associated layers of proteins adsorbed on ENMs.^[32] However, the structural features and detailed composition of environmental coronas in aquatic ecosystems remain unknown. Whilst in theory, an analogous framework is expected to exist in the environmental corona, with greater heterogeneity in the structure and components, a wider range of molecules of different molecular weights and compositions are competing for the surface of the ENM.^[24,43] To date, the structure-activity relationships in environmental corona formation on ENMs is not well understood, as it is dependent on the physicochemical properties of ENMs and chemical reactivities of eco-molecules.

In addition to the intrinsic properties of ENM, the surrounding medium is another key factor forming the environmental corona on ENMs. Distinct from the stable (highly-buffered) physiological conditions, the hydrochemistry (e.g., pH, ionic strength, oxygen, temperature, and

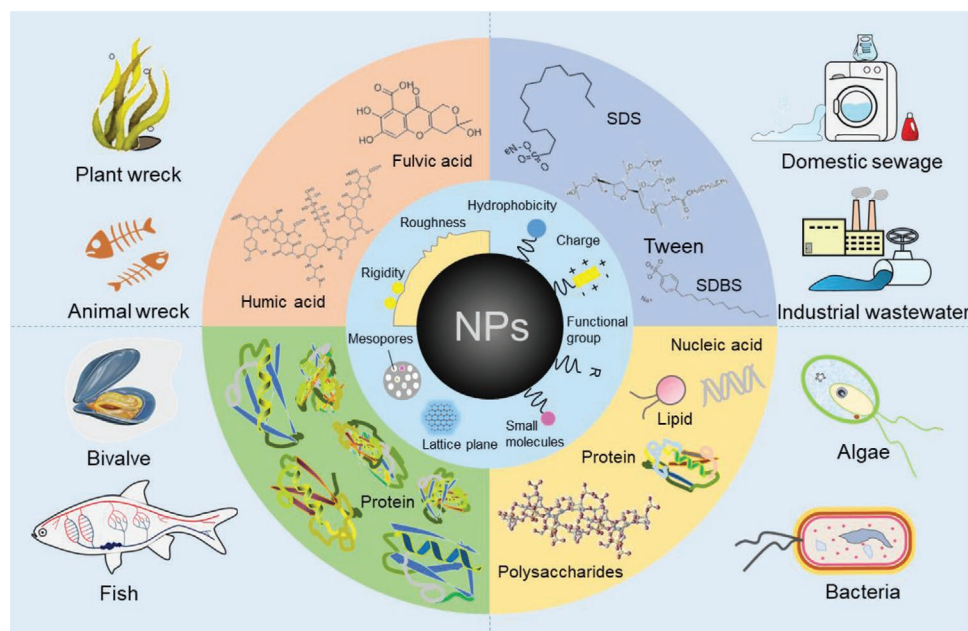


Figure 1. The characteristics of the environmental corona in aquatic environments. a) NOM is the decomposition products of animals and plants. HA and FA are the two major components of NOM. b) The secreted proteins (e.g., mucus proteins secreted by bivalves) and body fluid protein (e.g., plasma proteins of fish). c) EPS secreted by microorganisms (e.g., bacterial, algae). d) Surfactants released from detergents (e.g., SDS), food processing (e.g., Tween), cosmetics and other aspects.

macromolecules) of environmental waters fluctuates with spatio-temporal conditions depending on the water source (e.g., groundwater, lake water, seawater, and wastewater). Many abiotic and biotic transformation^[36,38,44,45] processes undergone by ENMs, such as oxidation, sulfuration, reduction, and dissolution, strongly influence surface morphology and modify ENMs. Thus, the chemical affinity of macromolecules to the surface of ENMs changes during these transformation processes. As a result, the environmental corona is dynamic, and will continuously evolve along with adsorption, desorption, redox, and other chemical reactions, which are currently poorly understood. Individual ENMs probably are likely to possess a unique fingerprint of an environmental corona, although naturally seasonal and temporal variations in biomolecule abundance may be of influence. On the other hand, ecological macromolecules can take part in the transformation process of ENMs.^[38,44]

Following corona formation and transformation, the surface free energy, repulsive forces, and steric hindrance of ENMs is significantly altered, determining the stabilization or destabilization of ENMs in aqueous medium, and subsequently influencing their environmental fate, bioavailability, and toxicity in aquatic ecosystems.^[36] ENMs may encounter various substances in water, such as NOM from plant or animal degradation, EPS secreted by microbes and plankton, body fluid proteins in fish, surfactants in wastewater, or other undefined chemicals (Figure 1). Based on the chemical characteristics of macromolecules and their natural or anthropogenic sources, we elaborate on the association and interplay at the eco-nano interface between ENMs and four main groups of substances in aquatic ecosystems; NOM, EPS, proteins, and other substances such as surfactants.

2.2. Typical “Environmental Corona” in Aquatic Ecosystems

2.2.1. Natural Organic Matter

NOM from internal and external sources is ubiquitous in natural aquatic ecosystems. They mainly consist of carbon, oxygen, hydrogen, nitrogen, and sulfur. Internal sources of NOM are mainly metabolites from aquatic organisms and decomposition products form the decay process, while external sources are mainly pollutants produced by anthropogenic activities.^[46] NOM is composed of hydrophobic and hydrophilic components. The latter is the major component comprised mainly of humic substances (HS), which account for 40–80% of total organic matter.^[41] HS may be divided into humic acid (HA) and fulvic acid (FA), based on their solubility at different pH levels.^[41] HA is more hydrophobic and less soluble compared to FA, containing more reactive functional ligands such as carboxyl and phenolic groups as well as a higher carbon and nitrogen content.^[47] Additionally, NOM also contains a diverse range of other components such as proteins, polysaccharides, lipids, and other organic matter.^[37] These components contain abundant chemical functional groups (e.g., sulfhydryl and aromatic groups),^[48–50] generating the strong binding capacity of NOM for ENM surfaces. As the interactions between NOM and ENMs have previously undergone a comprehensive review,^[37,41] it will only be briefly introduced in this section.

The adsorption process is determined by the chemical characteristics of NOM and the physicochemical properties of the ENMs. For example, different isolated NOM fractions with varied molecular weight distributions and aromaticity exhibit diverse interactions with Au NPs.^[51] The surface adsorption of HA on Al₂O₃ NPs was also reported to closely correlate with the

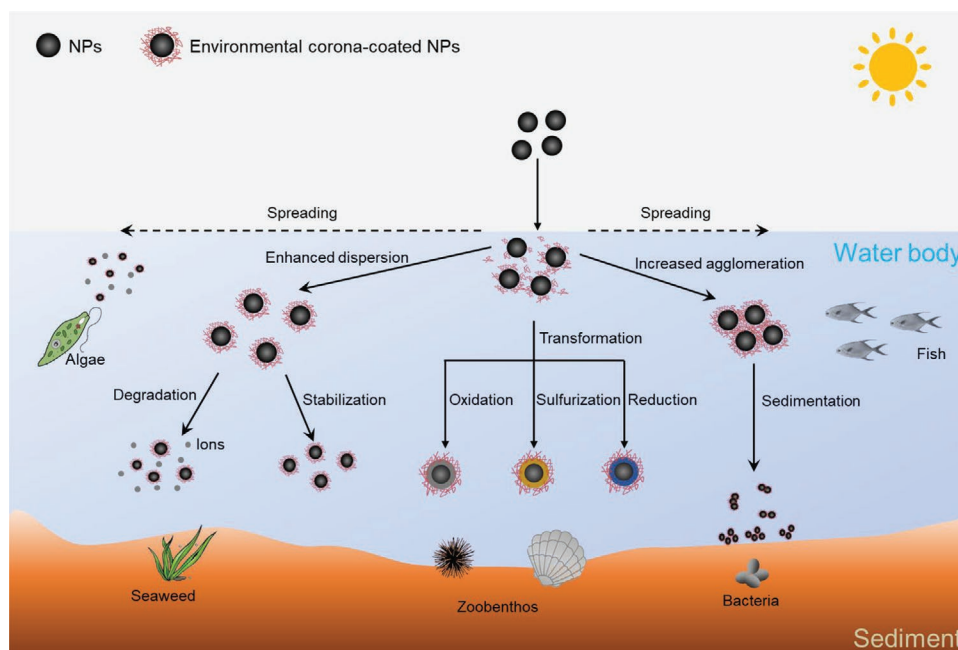


Figure 2. The effects of environmental corona on the environmental behaviors (e.g., dispersion, degradation, stabilization, agglomeration, transformation, spreading) of ENMs in aquatic environments.

polarity and chain length of HA components.^[52] On the other hand, adsorption is dependent on the intrinsic properties of ENMs, such as their size and surface.^[53–55] The adsorption of HA and FA on SiO₂ NPs is strongly size-dependent, and 20 nm SiO₂ NPs were found to have a significantly higher density of active sites compared their 100 and 500 nm counterparts, due to their large specific surface area.^[53] Surface properties, such as the presence of modification ligands, are also crucial to NOM adsorption onto ENMs. For instance, whilst HA could replace the surface ligand in stabilizing Au NPs when it was β -D-glucose, it would overlap on the surface when the stabilizer was citric acid.^[54] HA adsorption could cause the desorption of sodium dodecyl sulfate (SDS) from multi-walled carbon nanotubes (MWCNTs).^[55] Due to its complexity, the characterization of NOM itself and the NOM corona continues to be a substantial challenge, requiring high-resolution and high-precision analytical techniques.^[56] Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is a powerful tool that offers a feasible method to identify chemical information from the NOM corona. Utilizing FT-ICR-MS, the NOM corona on Ag NPs was found to be rich in N and S-containing compounds, and varied with the composition of the original NOM. However, its molecular weight, degree of unsaturation, and oxygenated groups were closely linked to the NOM used.^[57]

The NOM corona on ENMs is also highly sensitive to hydrochemistry parameters, such as pH and alkalinity. It has been reported that conformational changes in HA occurred on the surface of ferromagnetic (γ -Fe₂O₃) NPs at different pH values.^[58] At pH 9, HA had a stretched conformation, enhancing the colloidal stability of γ -Fe₂O₃ NPs through long-range electrosteric stabilization. However, strong destabilization of the HA-coated γ -Fe₂O₃ NPs was observed at pH 5 with a decrease in electrostatic repulsion. In another study, the

impact of HA on CeO₂ NPs was found to be more complicated in the presence of Ca²⁺ than K⁺.^[59] Irrespective of K⁺ concentration, HA drastically reduced the agglomeration of CeO₂ NPs. At low Ca²⁺ concentrations (0.004 M), HA could inhibit the agglomeration of CeO₂ NPs, whilst it promoted agglomeration at high Ca²⁺ concentrations (0.08 M), possibly because of the changes in steric repulsion and bridging attraction between the HA-coated CeO₂ NPs.

Following the formation of an NOM corona, there may be alterations to specific environmental behaviors of ENMs (e.g., agglomeration, stabilization, transformation, dissolution, and degradation),^[37,41] as the NOM corona may boost the attractive or repulsive forces between ENMs (Figure 2). For instance, Suwannee River FA, Suwannee River HA, alginate, and bovine serum albumin (BSA) increased the colloidal stability of MnO₂ NPs in the presence of Na⁺, Mg²⁺, and Ca²⁺ ions.^[60] As a semi-rigid globular macromolecule, the Suwannee River HA at a realistic environmental concentration (≥ 5 mg L⁻¹) could also induce significant disaggregation of large sub-micron TiO₂ ENM agglomerates.^[61] In addition, the dissolution of ZnO NPs was promoted by the HA coating at alkaline pH (9.0 or 11.0). This is potentially due to the polydentate complexing of HA and the availability of a greater number of functional groups for complexation.^[62] In contrast, HA did not significantly affect the dissolution of ZnO NPs at acidic pH (6.0, 3.0, or 1.0), which may reduce the activity and accessibility of functional groups to adsorb to ZnO NPs. The dissolution rate constant of ZnO NPs was positively related to the aromatic and carbonyl carbon content of NOM, and negatively related to its hydrogen/carbon ratio and aliphatic carbon content. This indicates that the aromatic carbon content of NOM was key in the dissolution process of ZnO NPs.^[63] Similarly, Suwannee River NOM was able to stabilize zero-valent

Cu NPs, increasing the available surface area, which in turn accelerated the dissolution process.^[64] However, the dissolution and agglomeration of NOM-stabilized Cu NPs may be altered by the addition of Ca^{2+} or Mg^{2+} , which is dependent on pH conditions.

2.2.2. Extracellular Polymeric Substances

Relative to the dominant organic acids in NOM such as HA and FA, EPS secreted by microorganisms (i.e., archaea, bacteria, algae) primarily contain polysaccharides and proteins. These account for approximately 70–90% of the total content, supplemented by lipids, nucleic acids, and other substances.^[65] Although polysaccharides are considered the major component, the chemical composition of EPS is highly heterogeneous and has not been well-characterized. Biofilm matrices are formed largely by EPS, providing a three-dimensional architectural framework to protect microorganism communities and facilitate their activity such as nutrient sequestration, cell communication, and gene exchange.^[66] Meanwhile, EPS may mediate the trophic transfer of ENMs in aquatic microorganisms, which may also be utilized for antimicrobial purposes.^[67]

Due to the excellent performance and wide application of ENMs as antibacterial agents,^[68] pesticides,^[69] and fertilizers,^[70] their interaction with EPS is a prerequisite for the exposure of ENMs to microbial communities in water systems. The biosynthesis of ENMs by microorganisms is regarded as a green and eco-friendly technology.^[71] The biopolymers of EPS are readily adsorbed, forming a corona on ENMs and mediating further interactions with microorganisms. As reported for wastewater, the surface of biogenic Se NPs was coated with an EPS layer, which governed the surface charge, colloidal properties, and environmental fate of Se NPs.^[72] Following purification, the carbohydrate, protein, humic-like substances, and DNA concentrations from this EPS layer were determined to be 313.8 ± 3.5 , 144.1 ± 2.1 , 158.2 ± 2.3 , and $4.6 \pm 0.8 \text{ mg g}^{-1}$ in biogenic Se NPs, respectively.^[72] Recently, a $\approx 3 \text{ nm}$ thick corona was observed on the surface of Au NPs produced by the arsenic-reducing bacterial strains, *Pantoea sp.* IMH, which mainly consists of membrane proteins, lipoproteins, and phospholipids.^[73] On double-walled carbon nanotubes (DWNs), 10 and 174 kDa protein-like polymers were identified as the predominant adsorbed components of EPS secreted by *Nitzschia palea*.^[74] In terms of the binding strength with microbial cells, the EPS from algal aggregates may be fractionated into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS).^[75] Spectral analysis has revealed that tryptophan and humic-like components in TB-EPS exhibit higher binding capacities to CuO NPs and Fe_3O_4 NPs than those in LB-EPS.^[75] Moreover, protein-like substances (N–H and C–N in amide II), and secondary carbonyl groups (C = O) in carboxylic acids of EPS were demonstrated to be important binding sites on ZnO and SiO_2 NPs.^[76]

The original EPS mixture may be separated into multiple fractions based on molecular weight or other parameters, as an alternative approach to determine the affinity of various EPS constituents for ENMs. This enables the assessment of the interaction mechanisms of different fractions with

ENMs. Based on this approach, the bulk cyanobacterial EPS matrix ($<0.45 \mu\text{m}$) has been divided into high molecular weight (HMW; $1\text{--}0.45 \mu\text{m}$) and low molecular weight (LMW; $<1 \text{ kDa}$) fractions.^[77] The HMW-EPS possessed stronger adsorption capacity on TiO_2 NPs than LMW-EPS, as a result of its higher aromaticity and richness of autochthonous protein-like substances compared to LMW-EPS. These findings suggest that the formation of the EPS corona is molecular weight and aromaticity dependent. Very recently, the soluble EPS, LB-EPS and TB-EPS, from activated sludge were extracted and compared for the transformation of Ag NPs.^[78] Results showed that the presence of all EPS fractions reduced the agglomeration rate of Ag NPs with NaNO_3 and low concentrations ($0.05\text{--}10 \text{ mM}$) of $\text{Ca}(\text{NO}_3)_2$, due to increased steric repulsion. Among the three EPS fractions, LB-EPS could more effectively stabilize Ag NPs, irrespective of the electrolyte, due to its lower hydrophilic components. As a natural semi-rigid linear biopolymer in EPS, alginate could weakly bind to Ag NPs and reduce the dissolution rates.^[79] The presence of alginate and flocculent sludge-derived EPS also enhanced the stability of CuO NPs; this was attributed to electrostatic stabilization combined with steric repulsion.^[65] Furthermore, several studies have focused on interactions between ENMs and phytoplankton-derived EPS, which are likely to have features distinct from those of bacterium-derived EPS.^[80] For instance, the cell surface-bound and soluble EPS from *Chlorella pyrenoidosa* (*C. pyrenoidosa*) exhibited different adsorption abilities and agglomeration effects on anatase and rutile TiO_2 NPs.^[81]

The adsorption of EPS substances is highly dependent on the intrinsic physicochemical properties of ENMs and the relevant aqueous conditions. For instance, the surface functional groups and charges (e.g., SO_4^{2-} , $-\text{COO}^-$, $-\text{NH}_2$) of polystyrene (PS) NPs can directly affect the binding affinity of EPS.^[82] The negatively charged sulfated PS NPs and COO^- -PS NPs exhibit the strongest and lowest affinity to biofilms, while the association of positively charged amine PS NPs to biofilms was sensitive to ionic conditions. The adsorption of soluble EPS onto TiO_2 NPs was also dependent on the surface area, charge, and hydrophobicity, driven by electrostatic interactions and chemical bonding between the $-\text{COO}^-$ groups of soluble EPS and TiO_2 NPs.^[83] Additionally, the presence of EPS was found to influence the colloidal stability of TiO_2 NPs via the intermolecular bridging of dissolved EPS and steric effects.^[84,85] Recently, it has been shown that EPS from *Bacillus subtilis* promoted the agglomeration of goethite ($\alpha\text{-FeOOH}$) NPs in the absence of ions at pH 6. However, the addition of EPS at 1 mg L^{-1} inhibited the $\alpha\text{-FeOOH}$ NP agglomeration in NaCl (168 mM), NaNO_3 (304.9 mM), or Na_2SO_4 (126.2 mM) solutions. This indicates that the colloidal stability of $\alpha\text{-FeOOH}$ NPs was strongly influenced by EPS adsorption.^[86]

2.2.3. Proteins

In mammalian bodily fluids (e.g., blood), protein corona formed on ENMs has been investigated intensively to gain insight into underlying molecular mechanisms and biological functions due to its significant physiological role.^[17,18] Once a protein corona is formed, it endows a new identity to ENMs and

mediates in vivo behaviors and biological effects, such as translocation, distribution, metabolism, and immune response. For example, it is well known that immunoglobulin and complement components in the protein corona of ENMs may trigger the clearance and immune response of immunocytes such as macrophages.^[87,88] At the nano-bio interface, the formation of the protein corona is dynamic, evolving with surrounding physiological conditions.^[89] There is a layer of a hard protein corona with strong binding affinity, long residence time, slow exchange time, and high conformational changes.^[90] It has been suggested that a soft protein corona also exists, consisting of looser contact and a rapidly exchanging layer of proteins with a low degree of conformational change.^[90] Unlike the protein corona under physiological conditions, the chemical composition of the environmental corona is highly heterogeneous with various types of macromolecules present in the water medium; this includes HS, polysaccharides, proteins, and lipids. Compared to other macromolecules, proteins have a more significant physiological role in mediating the biological interaction between ENMs and aquatic organisms. However, its characteristics in the environmental corona and its role in moderating the bioavailability and toxicity of ENMs remain poorly understood. When ENMs invade aquatic organisms, proteins inside cells or body fluids are adsorbed and form an analogous protein corona; this almost certainly influences the fate and hazard of ENMs. To date, most reported studies have focused on secreted and body fluid proteins from aquatic organisms. Recently, it has also been suggested that metabolites may be an important co-constituent alongside proteins.^[24]

Secreted proteins are particularly important for microorganisms in water systems, which have a wide range of biological functions from self-protection to reproduction.^[91,92] For example, cytochrome c (OmcA) is secreted by *Shewanella oneidensis* MR-1 (*S. oneidensis* MR-1), belonging to a bacterial extracellular protein.^[93] OmcA was found to stabilize hematite NPs, and its adsorption capacity was correlated to the protein-to-particle ratio, particle size (9, 36, and 112 nm) and salt concentration.^[93] Secreted proteins from *D. magna* have been reported to increase the uptake and toxicity of COOH- and NH₂-PS NPs, although it resulted in less efficient removal from the gut of *D. magna*.^[94] In another study, the secreted protein corona on Au NPs was found to contain at least 257 proteins, potentially reducing the agglomeration of Au NPs by shielding their surface attraction and detoxifying Au NPs to *D. magna*.^[95] This is likely as the mono-dispersed particles were less easily filtered and thus passed straight through with the water phase, as has been demonstrated for smaller PS particles compared to larger particles closer in size to the natural food sources of *D. magna*.^[96] Recently, proteins in the secretions of zebrafish were found to be adsorbed by graphene oxide nanosheets (GO NSs), further altering the morphology and toxicity of GO NSs.^[97]

On the other hand, proteins inside cells or bodily fluids (e.g., blood, cerebrospinal fluid, mucus, digestive juice, lymph, lysosomal fluid) for aquatic organisms play a more critical role in the cellular interactions and toxic effects of ENMs. In the hemolymph serum (HS) of *Mytilus galloprovincialis* (*M. galloprovincialis*), the protein coronas of NH₂-PS NPs, CeO₂ NPs, or n-TiO₂ NPs varied considerably.^[98,99] The putative

C1q domain-containing protein (MgC1q6) and Cu, Zn-superoxide dismutase (Cu, Zn-SOD) were identified as predominant components in the protein coronas of NH₂-PS NPs and CeO₂ NPs, due to differing surface properties.^[98,99] In the gill mucus of *Mytilus edulis*, the high-abundance extrapallial protein had barely adsorbed on SiO₂ and n-TiO₂ NPs because of its high histidine content and difficulty unfolding on the surface of NPs.^[98] In addition, protein corona showed a specific recruitment pattern according to the NP oxide (TiO₂ versus SiO₂) or crystal structure (anatase TiO₂ versus rutile TiO₂).^[100] Inside intact rainbow trout (*Oncorhynchus mykiss*) gill cells (RTgill-W1), a total of 383 different proteins were identified in the protein corona of Ag NPs, associated with cell membrane adhesion, uptake, vesicle trafficking, and stress response.^[101] Similarly, 326 adsorbed proteins were identified on ZnO NPs in the serum of juvenile *Cyprinus carpio*, related to acute-phase response signaling, liver and retinoid X-receptor activation, and intrinsic and extrinsic prothrombin activation.^[102] Additionally, in fish plasma, it has been reported that fish sex could affect the formation of the protein corona with sex-specific proteins and different corona thicknesses and surface charges.^[103,104] These findings suggest that proteins in the environmental corona may participate in diverse biological processes and promote the increased toxicity of ENMs. For instance, utilizing *Eisenia fetida* celomic protein (EfCP) as a native repertoire and fetal bovine serum (FBS) as a non-native reference, the importance of corona proteins in the recognition of Ag NPs by phagocytic or non-phagocytic cells has been compared.^[105] The results revealed that EfCP-coated Ag NPs had significantly greater accumulation potential in the immune effector cells when compared to FBS-coated Ag NPs. This indicates that EfCP facilitated the interaction between Ag NPs and phagocytic cells.

2.2.4. Other Substances

In addition to substances from natural sources, many artificial chemicals, such as surfactants, are continuously discharged into aquatic ecosystems. Surfactants are widely used in detergents, textile printing and dyeing, food processing, and other anthropogenic activities, leading to their universal presence in water bodies.^[106] The surfactant typically possesses a hydrophilic head and a hydrophobic tail, and may be classified as cationic, anionic, zwitterionic, and nonionic surfactants. Anionic, zwitterionic, and nonionic surfactants (e.g., sodium dodecylbenzenesulfonate (SDBS), cetyl betaine, and glycerol monostearate), have been shown not to affect the dissolution and sulfidation of silver nanowires (Ag NWs). In contrast, cationic surfactants (e.g., cetyltrimethylammonium bromide, cetyltrimethylammonium nitrate, and benzyldimethyldodecylammonium nitrate), have significantly promoted these processes in aquatic ecosystems.^[107,108] SDS may increase the release of Ag NPs from the laundry washing cycle into wastewater streams because of the increased colloidal stability arising from adsorption, resulting in a greater number of negative surface charges on the Ag NPs.^[107] Indeed, surfactants will impact the interactions between ENMs and aquatic organisms, and may also influence the unfolding of proteins in the corona,^[109,110] and/or their and other biomolecule displacements.^[111] However, very few studies have explored

the impact of synthetic surfactant constituents in the corona on ENM toxicity. A recent study compared the effects of Tween 60 and a biosurfactant in microalgae exudate on the ingestion of polyethylene (PE) particles by *D. magna*.^[112] The results showed that whilst the biosurfactant significantly increased the PE particle uptake, Tween 60 did not, indicating that the nature of the surfactant had a strong influence on particle ingestion. A synergistic toxicity was observed when TiO₂ NPs and disodium laureth sulfosuccinate coexisted, reducing the lysosomal membrane stability in the digestive cells of mussels.^[113]

Moreover, the exposure of environmental pollutants may be reinforced by adsorption to ENMs, increasing their potential risk to the ecosystem.^[114] As the presence of pharmaceuticals and personal care products (PPCPs) is increasing in domestic sewage, it is suggested that PPCPs may compete with NOMs for adsorption on single-walled carbon nanotubes (SWCNTs).^[115] Antibiotics, bisphenol A, tetrachlorodibenzo-p-dioxin, and other pollutants have been reported to be enriched in ENMs; thus, they are likely to induce synergistic or antagonistic effects in aquatic organisms.^[24,116–118] However, the bioavailability of ENMs may also be affected by the presence of these chemicals.^[119]

3. Ecotoxicological Effects

The latent hazards of ENMs on aquatic organisms have been investigated intensively;^[27–29] however, most reported studies focus on ENMs in an “ideal environment” as developed for regulatory testing where the goal is simply to rank chemicals on their toxicity. Such approach is unlikely to reflect their status in actual aquatic ecosystems. The presence of environmental corona alters the bioavailability of pristine ENMs and mediates interactions between environmentally transformed ENMs and aquatic organisms at different trophic levels (e.g., bacteria, algae, crustacea, and fish), as shown in **Figure 3**. Eventually, ENMs may be transported through the food chain and pose unknown ecological and human health risks.

3.1. Bacteria

As a member of the aquatic organism community, bacteria are a key link in the food web and play an important role in the bioaccumulation, biotransformation, and trophic transfer of ENMs. Based on the environment, bacteria may be divided into benthic, planktonic, and epiphytic communities.^[120] During the process of wastewater treatment, bacteria are the main constituents of activated sludge, which can quickly adsorb and degrade wastewater constituents.^[121]

There is a great deal of literature concerning the toxicological effects of ENMs on bacteria and biofilms, given that ENMs have been widely applied for antibacterial purposes.^[68] ENMs can reduce the growth, viability, and survival rate of bacteria; however, the effect of acquired environmental corona on the microbial toxicity of ENMs is debatable. HA may depress the toxicity of Ag NPs to *Pseudomonas fluorescens* (*P. fluorescens*), and increased its growth by approximately 20–25%.^[122] NOM from the Suwannee River may also weaken the toxicity and

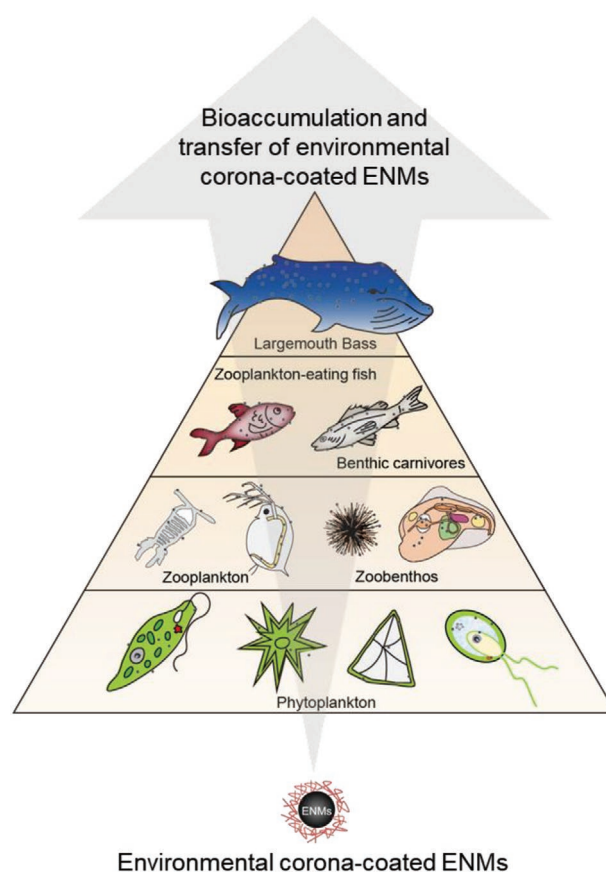


Figure 3. Schematic illustration of the bioaccumulation and trophic transfer of environmental corona-coated ENMs. The presence of environmental coronas (i.e., NOM, EPS, protein and surfactant) can mediate the ecotoxicological effects and trophic transfer of ENMs in food chain.

reduce the membrane damage of gram-negative bacterium *S. oneidensis* MR-1 induced by diamond nanoparticles (DNPs) in a dose-dependent manner.^[123] In another study, FA was found to completely mitigate the cell wall and membrane damage of *Escherichia coli* (*E. coli*) cells induced by CuO NPs, including prevention of the formation of ≈ 100 nm holes and the leakage of intracellular K⁺ ions that occurred in the absence of the FA corona.^[124] On the contrary, it has been reported that HA may enhance the toxicity of Pd@Ir NPs to gram-positive *Staphylococcus aureus* (*S. aureus*) bacteria and gram-negative *E. coli* bacteria.^[125] As secretions, EPS is able to protect bacteria against ENMs. For instance, the production of EPS and colonic acid by engineered *E. coli* protected the bacteria against Ag NPs.^[126] Moreover, as a commercial EPS polymer analog, the exogenous addition of xanthan at 100 mg L^{−1} significantly increased the viability of bacteria upon Ag NP treatment. The EPS-producing strain of *Sinorhizobium meliloti* was also demonstrated to have a higher survival rate than the parent strain when exposed to Ag NPs.^[126] In addition to secreted EPS, biofilms are able to protect planktonic bacterial cells (*P. fluorescens*) against positively charged polystyrene latex nanoparticles (PSL NPs); these generally covered the negatively charged bacterial surface and caused bacterial cell death.^[127] In another study, EPS (alginate) was found to protect the nitrification activity of ammonia-oxidizing

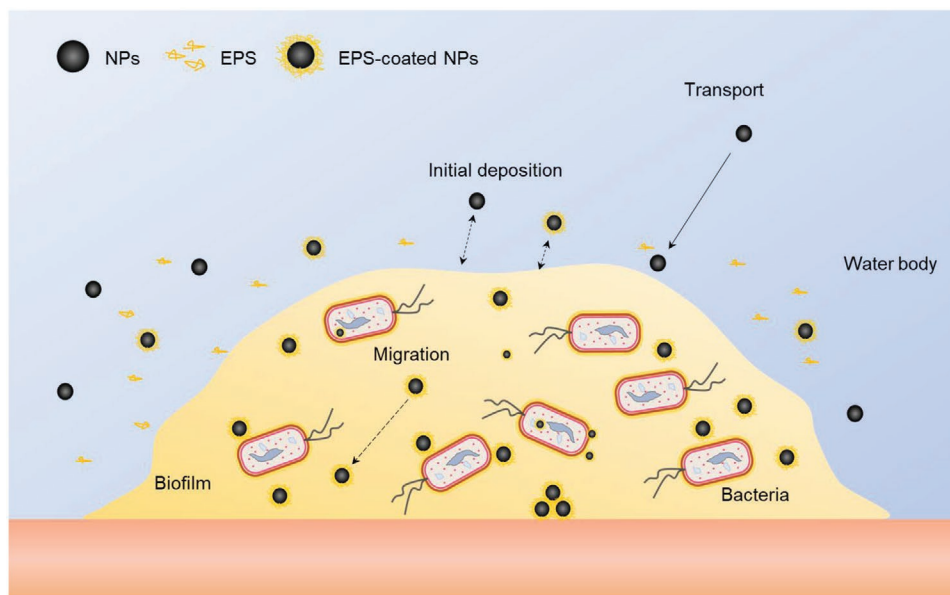


Figure 4. Representation of the stages (transport, initial deposition, and migration) involving ENMs transport phenomena within bacterial biofilms.

bacteria, *Nitrosomonas europaea* (*N. europaea*), against Ag NPs in a different manner compared with the protein (BSA). This indicates that EPS and protein-coatings play distinct roles in the adsorption of Ag NPs on bacterial biomass.^[79]

ENMs may also inflict harm onto bacterial activity and their morphological structures, including processes such as EPS secretion and biofilm development (Figure 4).^[128–131] To demonstrate interactions between ENMs and bacterial communities, EPS was removed from *E. coli* suspensions, and the toxicity of ZnO and SiO₂ NPs to *E. coli* was found to increase, indicating that the EPS matrix was critical to entrap NPs and prevent their contact with bacteria, enabling bacterial protection.^[76] TiO₂ NP treatment was able to increase bacterial diversity and alter the physiological activity and ecological function of periphytic biofilms.^[132] To resist the toxicity of TiO₂ NPs, the metabolic activities of periphytic biofilms were elevated in bacterial communities, including those of *Alphaproteobacteria*, *Gammaproteobacteria*, *Cytophagia*, *Flavobacteriia*, *Sphingobacteriia*, *Synechococcophycidae*, and *Oscillatoriothycidae*. Very recently, a reported study showed that high concentrations of ZnO NPs (>30 mg L^{−1}) significantly inhibited biofilm formation of *Pseudomonas putida*,^[133] while low concentrations (0.5–30 mg L^{−1}) promoted bacterial growth and biofilm formation. This was achieved by stimulating the expression of quorum sensing, lipopolysaccharide biosynthesis, and antibiotic resistance genes, and corroborating the increased protein and sugar content of the biofilm matrix. Moreover, the −OH and −NH₂ branches of hydroxyl and amine groups in EPS produced by bacteria were significantly affected by treatment with CeO₂ NPs.^[134]

3.2. Algae

Algae are generally divided into planktonic and benthic communities based on their ecological characteristics. They have a wide distribution and strong adaptability in aquatic ecosystems. Algae contain photosynthetic pigments such as chlorophyll and

can use photosynthesis to convert carbon dioxide and water into oxygen and sugars, playing a crucial role in material circulation and energy flow in ecosystems. Previous studies have demonstrated that ENMs have adverse effects on the growth, morphology, and photosynthesis of algae.^[135] In recent years, there has been increasing value placed on the importance of environmental corona for these hazards. For instance, with 5 mg L^{−1} dissolved HA or the surface saturation of HA, the half-maximal inhibitory concentration (IC₅₀) of TiO₂ NPs on *Chlorella* sp. increased from 4.9 to 18 mg L^{−1} or 48 mg L^{−1}.^[136] However, it was reported that low concentrations of HA (<5 mg L^{−1}) had little impact on the Ag NP-induced toxicity on the biomass of *Microcystis aeruginosa* (*M. aeruginosa*), whilst algal cells had higher photosynthetic activity when HA increased to 10 or 20 mg L^{−1}. This indicates that HA had actually alleviated the algal toxicity of Ag NPs at relatively high concentrations, and ENMs need to be fully covered for the corona to be effective.^[137]

Similar to the effects observed with bacteria, EPS secreted by algae can strongly interact with ENMs, mediate their contact opportunities in aqueous medium, mitigate ENM-induced toxicity on algae, increase algal survival, and induce morphological changes (e.g., plasmolysis and membranolysis).^[138–140] For example, exposure to 10 mg L^{−1} CuO NPs increased the thickness of the EPS layer on *C. pyrenoidosa* from ≈0.1 to 0.4 μm.^[141] Further characterization showed that both soluble and algal-bound EPS had significantly increased in comparison with the unexposed algae. ENMs may exhibit alterable toxicity at different algal growth phases, due to varied EPS production. As EPS concentration more than doubled in cultures along with the growth of *Chlamydomonas reinhardtii* (*C. reinhardtii*), Ag NPs lead to reduced toxicity in later stages than during the earlier stages of algal growth.^[142] Similarly, CeO₂, CuO, and ZnO NPs are able to alter the production and composition of EPS secreted by *M. aeruginosa*, where polysaccharides were the predominant portion affected compared with proteins and HA.^[143] Once EPS was removed from the green algae cell surface

(*Chlorella vulgaris* (*C. vulgaris*)), Ag NPs depressed chlorophyll a activity in algae cells more so than in those with EPS, suggesting that EPS may alleviate photosynthetic toxicity of Ag NPs through the prevention of surface adhesion and the resultant shading.^[144]

3.3. Daphnia Magna

As zooplankton, the water flea, *D. magna*, may be found in freshwater and brackish habitats such as lakes, rivers, and pools. They are vulnerable to predation by fish and other invertebrates. *D. magna* are filter feeders consuming bacteria, fungal spores, and algae, being able to filter particulates less than 50 µm in diameter for ingestion from surrounding water.^[145] Additionally, they are also a widely used indicator organism to assess the ecological risk of pollutants in aquatic ecosystems. Unlike unicellular organisms such as bacteria and algae, particulate matter may be ingested and assimilated in the gastrointestinal tract of *D. magna*, helpful when assessing the bioavailability and biokinetic mechanism of exogenous particulates such as ENMs.^[146–148]

The mortality, survival rate, feeding rate, and locomotion of *D. magna* may be impacted by ENMs.^[149–151] Again, most studies have ignored the presence of an environmental corona on ENMs, leading to proposals of theories that deviate from what is actually occurring in natural ecosystems. Thus, current knowledge must be updated to ground-truth the research. For this purpose, the effects of seven NOMs (i.e., HA, seaweed extract, Suwannee River NOM, Suwannee River HA, Suwannee River FA, Leonardite, and Pahokee peat), on the acute toxicity of TiO₂ NPs toward *D. magna* were examined.^[152] Results showed that the toxicity of TiO₂ NPs decreased with increasing NOM concentration and was independent of the NOM type used and the specifics of the TiO₂ NPs tested. More importantly, the aromaticity and hydrophobicity of NOM seemed to play a critical role in modulating the toxic effects of TiO₂ NPs. In the presence of Pony Lake FA (PLFA), the toxicity of Ag NPs to *D. magna* decreased to almost 70%.^[153] It is worth noting that the environmental level of NOM (e.g., 10 mg L⁻¹ HA) is considered sufficient to protect *D. magna* against PS NPs, indicating that the effect of NOM on the toxicity of ENMs should not be underestimated in real-life scenarios.^[154] In addition, HA may increase the polarity of C₆₀ NPs and decrease their uptake by *D. magna*. This also slightly facilitated the depuration of C₆₀ NPs in *D. magna*, potentially due to the increased mobility of HA-coated C₆₀ NPs.^[155] However, secreted proteins from *D. magna* were found to induce the destabilization of PS NPs, inhibit their uptake, and decrease the half-maximal effective concentration (EC₅₀) of COOH-PS and NH₂-PS NPs.^[94] Once ingested, the secreted protein-coated PS NPs remained within the gastrointestinal gut of *D. magna* for longer, affecting their ability to feed on algae; this is indicative of the occurrence of a secondary effect.

In addition, other types of natural macromolecules have been reported to alter interactions between ENMs and *D. magna*.^[156] For instance, chitosan, as a representative substance in aquatic ecosystems, could increase the stability of CeO₂ NPs, while alginate enhanced their agglomeration and sedimentation rates. Consequently, alginate-coated CeO₂ NPs triggered oxidative

stress in *D. magna*, whilst behavioral assays showed that chitosan-coated CeO₂ NPs induced hyperactive behavior such as increased average swimming speed and acceleration.^[156]

3.4. Bivalves

Bivalves represent a unique target group for ENM exposure as an aquatic invertebrate that resides in the water column and sedimentary compartments.^[98] As good biological indicators, bivalves are widespread in all fresh, brackish, and salt water environments, and their easy retrievability and high sensitivity to environmental pollutants are beneficial in ecotoxicological studies. For example, the application of a battery of functional tests on *Mytilus* immune cells and hemocytes was demonstrated to be a powerful tool to screen the immunomodulatory effects of ENMs.^[98] Bivalves are also commercially harvested aquatic products as a food source for human consumption, implicating the latent exposure risks of ENMs through dietary uptake.

In aqueous media and biological fluids, the environmental corona has previously been identified as a key factor influencing the bioavailability, uptake, and toxicity of ENMs to bivalves. To explore the fate and toxicity of ENMs in seawater, the effect of NOM on ZnO and MnO₂ NPs on oyster larvae was investigated. The study demonstrated that NOM played a mitigating role in the toxicity of ZnO NPs by lowering the bioavailability of released Zn²⁺ ions through NOM complexation.^[157] In the absence of NOM, most larvae failed to develop calcified structures. The gills and digestive glands of bivalve species are the most sensitive organs to ENMs, as evidenced by the occurrence of immune system activation and oxidative stress in numerous studies.^[158,159] To gain insight into the underlying mechanism, protein coronas formed on five TiO₂ and one SiO₂ NPs in the secreted gill mucus from *Mytilus edulis* were identified, being able to defend itself against xenobiotics in aquatic environments.^[100] The results showed that the extrapallial protein (EP), one of the most abundant mucus proteins, was absent from the protein coronas. Moreover, although the majority of their protein coronas were similar, a few proteins in the corona had a specific recruitment pattern on TiO₂ or SiO₂ NPs. For example, major vault protein and histone, indicating that varied biological interactions and toxic effects of ENMs on gills may be mediated by unique corona compositions. The immune system in shellfish represents another sensitive target of ENM exposure. In the presence of HS, NH₂-PS NPs have been found to cause more serious cellular damage and reactive oxygen species (ROS) production in *M. galloprovincialis* hemocytes than in the absence of HS, due to recognizable biological identity with MgC1q6 in their protein corona.^[160] In addition, the negative charge and rounded shape of CeO₂ NPs adsorbed Cu, and Zn-SOD in HS had triggered higher changes in stress and immunological responses from *M. galloprovincialis* hemocytes. In contrast, the neutral and well-faceted CeO₂ NPs did not show either corona formation or significant immune responses.^[99]

3.5. Fish

In aquatic systems, ENMs may be directly adsorbed or ingested by fish, or transported to fish along the food chain

from organisms at lower trophic levels (e.g., bacteria, algae, and zooplankton);^[161,162] this poses health risks to consumers. Knowledge regarding the interactions between corona-coated ENMs and fish is limited, although it has been reported that ENMs may affect the embryo hatching rate, mortality, teratogenicity, neurotoxicity, reproductive toxicity, and genotoxicity of fish.^[163–165]

To elucidate possible toxicological mechanisms involved in these outcomes, it is necessary to focus on the corona formed on ENMs in aquatic ecosystems or in organisms such as fish body fluids. Recently, the impact of HA, alginic acid, BSA, and various forms of dissolved organic matter (DOM) on the toxicity of ZnO NPs to embryonic *Danio rerio* (*D. rerio*) was compared.^[166] The study showed that HA could most effectively mitigate the toxicity and hatching rate reduction caused by ZnO NPs.^[166] In addition, the effects of HA on the developmental toxicity of Ag NPs, SWCNTs, CdSe NPs, and ZnO NPs on *D. rerio* have been examined.^[167] Results showed that whilst the overall survival rates were not mitigated by HA, the addition of HA could restore ZnO NP-induced hatching inhibition and reduced head-tail angle in developing *D. rerio*. ZnO NPs may induce abnormal phenotypes in the nervous and vascular systems of developing *D. rerio* and subsequent generations, such as secondary motoneurons axonal projections, dorsal root ganglion development, and blood vessel development. However, these abnormal phenotypes may be mitigated by DOM, indicating that the rich organic material in natural waters can substantially reduce the risk of ENMs.^[168] Another reported study demonstrated that HA did not alter the biological fate of Ag NPs in adult *D. rerio*, rather, decreasing bioavailability, organ accumulation, toxicity, and fish mortality.^[169] In the presence of HA and FA, the penetration of Ag NPs into internal tissues following oral ingestion by *D. rerio* may be markedly inhibited, further decreasing bioaccumulation and affecting the tissue distribution of Ag NPs.^[170] In addition, the EPS-coating of Se NPs led to a tenfold reduction in toxicity to *D. rerio* compared to BSA-coated Se NPs. The EPS had lowered bioavailability and toxicity by decreasing interactions between the Se NPs and fish embryos.^[171]

On the contrary, an environmental corona may also elevate the bioavailability of ENMs in fish.^[172] For instance, surfactants (i.e., polysorbate 20, polysorbate 80, and SDS) exhibited synergistic toxicity with Au NPs in embryonic *D. rerio*, probably due to the increased bioavailability of surfactants by transport and internalization of the Au NPs.^[173] Similarly, GO NSs coated with biological secretions in *D. rerio* culture water (e.g., small organic molecules, proteins, nucleotides, and mucopolysaccharides), induced more serious embryotoxicity in *D. rerio* with higher proportions of mortality and malformation.^[97] In addition, tail flexure, pericardial edema, and faster heartbeat were observed in GO NS-treated juvenile fish.

In fish blood, the adsorption of plasma proteins on the surfaces of ENMs may influence their in vivo behavior and biological effects. Plasma proteins may lead to the agglomeration of Cu₂O NPs and alleviate their hemolytic effect on the blood cells of the freshwater fish, *Carassius auratus* (*C. auratus*).^[127] Protein adsorption onto SiO₂ NPs has been found to be sex-specific with or without the egg yolk precursor protein, vitellogenin, which could alter the degree of NP uptake by immune

cells in *D. rerio* blood.^[104] These findings indicate that SiO₂ NPs with female biological identity may preferentially accumulate in the lymphoid and myeloid populations of the blood cells, irrespective of the fish gender. Analogously, the protein corona of Ag NPs in the plasma of smallmouth bass *Micropterus dolomieu* was affected by their sex, which may raise potential issues of reproductive toxicity related to ENM-acquired environmental coronas.^[103]

4. Interaction Mechanisms Underlying Differential Toxicity of Environmental Corona-Coated ENMs Relative to Pristine ENMs

In aquatic ecosystems, the acquired environmental corona change the fate of ENMs, resulting in very different ecological toxicological outcomes compared to their pristine counterparts because of altered interaction mechanisms. As is well known, ENMs can induce membrane damage, oxidative stress, organelle dysfunction, genetic dysregulation, immune response, enzyme inactivation, metabolic disturbance, and programmed or unprogrammed cell death.^[174–177] However, the influence of the environmental corona on these modes of action has not been thoroughly investigated. As shown in **Figure 5**, there are four major ways in which ENMs may interact with an aquatic organism; 1) adsorption to the surface (cell, organ, or body), 2) cellular internalization, 3) dissolution of ions from the NPs, and 4) nano-effects including interaction with key signaling pathways. These mechanisms may be enhanced or weakened by the corona coating.^[28] The potential impact of the environmental corona on various toxicological endpoints identified herein are explored in detail in subsequent sections, and summarized in **Table 1**.

4.1. Membrane Adhesion

The bioavailability of ENMs toward aquatic organisms is the premise of their toxicity, shaped by the physicochemical properties of ENMs, environmental transformation, and corona characteristics. For instance, some studies have reported that the bioavailability of ENMs was size-dependent and affected by colloidal stability.^[178–181] The membrane adhesion, internalization, and intracellular transformation of ENMs are strongly correlated with unique corona coating.^[88,182] The corona may shield the surface properties of pristine ENMs in aqueous medium and change the nano-bio interactions at interfaces, avoiding or facilitating the recognition of ENMs by cells.^[183]

The adhesion of ENMs to aquatic organisms is the first step in the toxicity process. In general, it is widely accepted that the phospholipid bilayer and membrane proteins of the cell membrane contain carboxylic, phosphate, and other acidic functional groups, which normally exhibit a negatively charged surface. Thus, ENMs with positive surface charges tend to result in greater adhesion to cell membranes.^[183] However, the formation of the environmental coronas on ENMs change their original surface

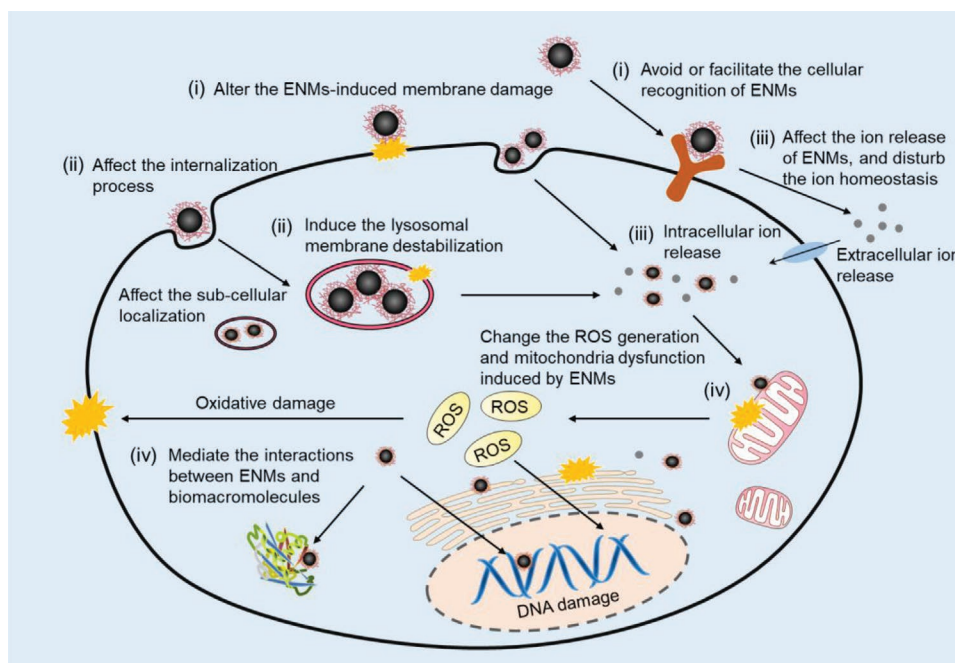


Figure 5. Altered toxicological mechanisms of ENMs by environmental corona. i) The presence of environmental corona can affect the attachment and adhesion of ENMs to the cell surface, alter the interactions between ENMs and cell membrane and result in differential cellular recognition; ii) The cellular internalization and sub-cellular localization of ENMs can be changed by macromolecules in environmental corona, due to modified surface properties; iii) Environmental corona may affect the extracellular or intracellular dissolution and ion release of ENMs, which can further disturb the cellular homeostasis and oxidative stress; iv) Environmental corona may mediate the interactions between ENMs and biomacromolecules (e.g., protein and DNA) or cellular components (e.g., mitochondria and nucleus).

charges and other synergistic surface properties, and determine the strength of ENM attachment to the cell wall or membrane.^[184] The adsorption of BSA or HA could cover the surface Si–OH/Si–O[−] groups on SiO₂ NPs and weaken their interaction with phospholipids.^[185] A recent report investigated the impact of NOM on the adsorption of DNPs functionalized with the polycation poly(allylamine HCl) to model biological membranes and the gram-negative bacterium, *S. oneidensis* MR-1.^[123] Their findings revealed that the concentration ratio of NOM-to-DNPs determined the surface charge and hydrodynamic properties of DNPs and altered the DNP attachment to bacterial membranes. In another study, Suwannee River NOM was able to either inhibit or promote the adsorption of Ag and C₆₀ NPs on the positively or negatively charged biomimetic lipid bilayers, depending on the type of NPs and NOM coatings.^[186] Moreover, the high propensity of membrane proteins have been highlighted to interact with TiO₂ NPs, which may play an important role on their membrane interaction process.^[187] However, to date, only very limited evidence has been provided to clearly display direct interactions or adhesion sites between environmental corona-coated NPs and real cell membranes, such as receptor-mediated binding.^[105]

4.2. Membrane Damage

As the first protective barrier, the cell membrane ensures the integrity of cells and participates in various cellular activities such as substance transfer. After attachment to the cell membrane, ENMs may more or less affect the structure and function

of the phospholipid bilayer and membrane proteins.^[88,188] Some ENMs (e.g., GO and Ag NPs) have already been demonstrated to impair the permeability and fluidity of the cell membrane, disorder the expression of membrane proteins, and alter membrane morphology.^[88,188–190] Additionally, the activation of membrane receptors may cause the abnormal regulation of their downstream signaling pathways, leading to further harmful consequences.^[188] Due to the formation of an environment corona, ENM-induced membrane damage may be mitigated or aggravated depending on the specific corona composition; however, the underlying mechanisms remain unclear.

Both HA and BSA are able to decrease NP-induced membrane damage compared to pristine SiO₂ NPs. HA adsorption causes more serious membrane gelation than BSA adsorption on SiO₂ NPs, due to the large number of functional groups in HA and their interactions with phospholipids through hydrogen bonds and hydrophobic effects.^[185] Similarly, HA-coated Al₂O₃ NPs inflict more serious membrane damage, including impaired integrity and fluidity of the cell membrane, while BSA-coated Al₂O₃ NPs adhered to the membrane without notable damage.^[191] A recent study quantitatively compared the thickness of various NOM-coating layers on Ag₂S NPs, in which thickness was found to be, in descending order: BSA (7.7 nm) > Suwannee River HA (2.5 nm) > alginate (2.4 nm) > Suwannee River FA (2.3 nm). Thickness was also negatively correlated with toxicity toward *E. coli*.^[192] Among these substances, BSA and Suwannee River HA were more effective in mitigating membrane damage induced by Ag₂S NPs by physically alleviating contact between NPs and the membrane. Moreover, the

Table 1. Biological effects and toxicological mechanisms of ENMs with or without environmental corona.

Organism	Macromolecules	ENMs	Altered biological effects		Toxicological mechanisms	Ref.
			Without corona	With corona		
Bacteria	<i>P. fluorescens</i>	Suwannee River HA	Ag NPs	Adversely affected bactericidal growth.	Mitigated bactericidal action.	HA-coating presented a physical barrier between the Ag NPs and the bacteria and prevented their interaction. [122]
	<i>S. oneidensis</i> MR-1	Suwannee River NOM	DNPs	Induced a comparable degree of membrane damage and toxicity.	Weaken the toxicity in a dose-dependent manner.	NOM reversed the charge of DNPs and eliminate their attachment to the membrane. [123]
	<i>E. coli</i>	FA	CuO NPs	Disruption of the bacteria wall and membrane.	Reduced both cell wall and membrane damages.	FA enhanced the electrostatic repulsion and hindered the physical contact between CuO NPs and bacterial membrane. [124]
	<i>S. aureus</i> <i>E. coli</i>	HA	Pd@Ir NPs	As potential antibacterial agents due to their distinctive oxidase-like activity.	Enhanced the bactericidal activity.	HA efficiently enhanced the oxidase-like activity of Pd@Ir NPs and promoted their cellular internalization. [125]
	<i>E. coli</i>	Suwannee River HA Suwannee River FA	WS ₂ NPs MoS ₂ NPs CdS NPs	Increased <i>E. coli</i> death.	Limited changes on the toxicity of CdS NPs, but decreased the toxicity of WS ₂ and MoS ₂ NPs.	HA and FA decreased the generation of •O ₂ ⁻ , •OH and ¹ O ₂ by WS ₂ NPs and MoS ₂ NPs, but promoted the generation of •O ₂ ⁻ by CdS NPs. [201]
	<i>E. coli</i>	EPS Colanic acid Xanthan	Ag NPs	Affected Colony-Forming Units (CFU) and survival percentage.	Resulted in better growth in terms of CFU and survival percentage.	EPS, colanic acid and xanthan promoted the agglomeration of Ag NPs, and trapped the Ag NPs outside the cells. [126]
	<i>P. fluorescens</i>	EPS	PSL NPs	NPs adhered directly to the cell surface, leading to death.	Resistance to NP toxicity, and most bacterial cells were viable.	Biofilms formed structured communities of bacterial cells embedded in self-produced EPS, which provided resistance against antibacterial agents. [127]
	<i>N. europaea</i>	BSA Alginate	Ag NPs	Reduced the bactericidal nitrification activity.	Both BSA and alginate increased the nitrification activity.	BSA reduced the toxicity by chelating the Ag ⁺ ions released from Ag NPs, while alginate reduced the toxicity via surface coating on Ag NPs and reducing their dissolution. [79]
	<i>E. coli</i>	EPS	ZnO NPs SiO ₂ NPs	Reduced the survival rate of <i>E. coli</i> .	Increased the survival rate of <i>E. coli</i> .	The EPS matrix sequestered the ZnO NPs and SiO ₂ NPs through the formation of NP-EPS complexes. [76]
	<i>E. coli</i>	Suwannee River HA Suwannee River FA BSA Alginate	Ag ₂ S NPs	Induced cell membrane damages.	The thickness of NOM coating layers was negatively correlated with the toxicity of Ag ₂ S NPs.	BSA and Suwannee River HA were more effective on alleviating the Ag ₂ S NPs induced membrane damages through physically alleviating the contact between NPs and membrane. [192]
Algae	<i>Chlorella</i> sp.	HA	TiO ₂ NPs	Inhibited the algal growth.	Significantly alleviated the algal toxicity of TiO ₂ NPs.	HA prevented the adhesion and agglomeration of TiO ₂ NPs on algae cells, and inhibited the production of intracellular ROS. [136]
	<i>M. aeruginosa</i>	HA	Ag NPs	Reduced microalgae biomasses and photosynthetic activity.	Mitigated the toxicity of Ag NPs.	HA reduced the release of Ag ⁺ ions from Ag NPs and their direct contact with algae cells. [137]
	<i>C. reinhardtii</i>	EPS	Ag NPs	Higher toxicity in earlier stages of algal growth.	Lower toxicity in later stages of algal growth.	The concentration of EPS increased more than two times in cultures along with the algal growth, and mediated inactivation of NPs and ionic silver. [142]

Table 1. Continued.

Organism	Macromolecules	ENMs	Altered biological effects		Toxicological mechanisms	Ref.
			Without corona	With corona		
<i>C. vulgaris</i>	EPS	Ag NPs	Led to the toxicity of photosynthesis.	Protected chlorophyll and alleviated the toxicity of photosynthetic.	EPS displayed extraordinary barrier effect on Ag NPs and Ag ⁺ ions, and reduced the accumulation of silver in algae.	[144]
<i>M. aeruginosa</i>	Suwannee River FA	CuO NPs	Induced DNA damages.	Enhanced ROS production and DNA damages induced by CuO NPs.	Suwannee River FA promoted the dissolution of CuO NPs and increased the amount of small-sized CuO NPs and Cu ²⁺ ions, which increased the cellular internalization of CuO NPs.	[196]
Crustacean	<i>D. magna</i>	HA	Caused acute toxicity	The toxicity decreased up to a factor of >18 with increasing NOM.	NOM adsorbed to TiO ₂ NPs surface, resulting in steric repulsion forces among NOM coated-NPs, that subsequently reduced the NPs-related acute toxicity.	[152]
	Seaweed extract	TiO ₂ NPs				
	Suwannee River	Ag NPs	Induced adverse effects by 48 h acute toxicity test.	The adverse effects of Cit-Ag NPs and BPEI-Ag NPs were reduced by about 70% and 60%, respectively.	The amount of free dissolved Ag ⁺ ions released from Ag NPs in the presence of Pony Lake FA was lower than their concentration in the absence of Pony Lake FA, due to complexation of free dissolved Ag with Pony Lake FA fractions.	[153]
	NOM Suwannee River HA					
	Suwannee River FA					
	Leonardite					
	Pahokee Peat	PS NPs	The 100% mortality after a 72 h treatment.	HA (50 mg L ⁻¹) subdued the PS NPs (400 mg L ⁻¹) toxicity effect completely.	The HA-coating changed the distribution of PS NPs in <i>D. magna</i> neonates and led to the alleviated toxicity.	[154]
	Pony Lake FA					
<i>D. magna</i>	Suwannee River HA	C ₆₀ NPs	The maximum C ₆₀ NPs uptake at 24h.	Slightly increased the removal efficiency of C ₆₀ NPs from <i>D. magna</i> .	HA decreased the uptake and increased the removal efficiency of C ₆₀ NPs, which might be due to the size effect, the altered polarity and increased mobility of C ₆₀ NPs.	[155]
<i>D. magna</i>	HA	Ag NPs	Induced higher toxic effects on the growth and mortality.	Enhanced the survival of the F0-F3 generations of <i>D. magna</i> .	Less toxic effects were observed, due to the reduction of ionic Ag arising from HA stabilization of the ENMs.	[216]
<i>D. magna</i>	HA	TiO ₂ NPs	Trans-generational reductions in sizes and tail losses/tail length reductions.	Reduced the morphological defects.	In HA-containing medium, the uptake of the total Ag by the daphnids was reduced.	[217]
<i>D. magna</i>	Secreted protein	COOH-PS NPs NH ₂ -PS NPs	Decreased the survival rate.	Lower EC ₅₀ and less removal efficiency.	A great amount of proteins adsorbing to the surface of NPs, causing the agglomeration of NPs and increasing their accumulation in <i>D. magna</i> .	[94]
<i>Ceriodaphnia dubia</i>	Suwannee River HA	Ag NPs	Led to the death.	Decreased the toxicity with the increasing concentration of HA.	The precise mechanisms of the decrease in toxicity for Ag NPs is unknown, but might be a function of complexation of Ag ⁺ with HA molecules and/or passivation of the NPs surface by HA. In addition, HA may mitigate the toxicity of Ag NPs by serving as a free radical scavenger.	[226]

Table 1. Continued.

Organism		Macromolecules	ENMs	Altered biological effects		Toxicological mechanisms	Ref.
				Without corona	With corona		
Bivalve	<i>Oyster larvae</i>	Suwannee River NOM	ZnO NPs	Induced oxidative stress.	Mitigated the toxicity.	NOM reduced the release of Zn ²⁺ ions from ZnO NPs.	[157]
	<i>M. galloprovincialis</i>	HS protein	NH ₂ -PS NPs CeO ₂ NPs	Changed cell functional parameters, such as the lysosomal membrane stability, oxyradical production, phagocytosis.	Induced more serious cellular damages.	The presence of MgClq6 in protein corona increased the generation of extracellular ROS. The Cu, Zn-SOD in protein corona triggered higher changes in stress and immunological response of hemocytes.	[99] [160]
Fish	<i>D. rerio</i>	HA Alginate acid BSA Suwannee River NOM Yukon River NOM	ZnO NPs	Reduced the hatch of embryos at 72 h post fertilization.	All macromolecules mitigated the ZnO NPs-induced toxicity.	The macromolecules changed the interactions between ZnO NPs and embryos and thus reduced the accumulation or precipitation of ZnO NPs on the chorion.	[166]
	<i>D. rerio</i>	HA Alginate acid BSA Milwaukee River NOM Yukon River NOM Suwannee River NOM	ZnO NPs	Induced neural and vasculature toxicity.	Mitigated the ZnO NPs-induced neural and vasculature toxicity.	The macromolecules decreased the interactions between ZnO NPs and the embryo.	[168]
	<i>D. rerio</i>	HA	Ag NPs	LC ₅₀ = 25.0 mg L ⁻¹ .	LC ₅₀ = 40.56 mg L ⁻¹ .	HA decreased the bioavailability and accumulation of Ag NPs in fish.	[169]
	<i>D. rerio</i>	Fish secretions	GO NSs	Induced death and malformation.	Induced more serious embryotoxicity.	GO NSs coated with secretions had smaller lateral sizes, more negative surface charges and lower aggregation state than pristine GONSSs, and tended to cover the embryos, inhibiting the oxygen and ion exchange.	[97]
	<i>C. auratus</i>	Plasma protein	Cu ₂ O NPs	Induced the hemolytic effect.	Alleviated the hemolytic effect to the blood cells.	The protein adsorption caused the aggregation of Cu ₂ O NPs in whole blood, alleviated the hemolytic effect and subsequently mediated the phagocytosis.	[227]
	<i>Tetrahymena pyriformis</i>	HA	TiO ₂ NPs	Induced a 25% of mortality rate.	Increased the vitality.	The internalization of HA-coated TiO ₂ NPs by cells decreased due to their higher negative charge intensity compared to bare TiO ₂ NPs.	[228]
Rotifera	<i>Brachionus plicatilis</i>	Natural NOM in seawater	COOH-PS NPs NH ₂ -PS NPs	Induced the death in the reconstituted sea water.	Decreased the toxicity.	NOM might significantly affect bioavailability.	[229]
Gastropoda	<i>Lymnaea stagnalis</i>	Suwannee River HA	Ag NPs	Efficiently assimilated by <i>Lymnaea stagnalis</i> .	Showed no effects on the bioaccumulation and toxicity.	HA affected the interactions between Ag NPs with membrane.	[230]

effects of two natural South African river water samples (Elands River (ER) and Bloubaan River (BR)) on the toxicity of ENMs have been compared.^[193] The results showed that high concentrations of ZnO NPs (100 and 1000 µg L⁻¹) induced a significant reduction in the membrane integrity of *Bacillus subtilis* in ER, being absent for BR. They also found that γ-Fe₂O₃ NPs showed no difference in both water samples, indicating that the unique physicochemical properties and substances of natural

aqueous media may have distinct corona compositions on different ENMs, generating different outcomes.

4.3. Cellular Internalization

Following membrane adhesion, ENMs may be internalized across the plasma membrane, accumulated inside cells, and

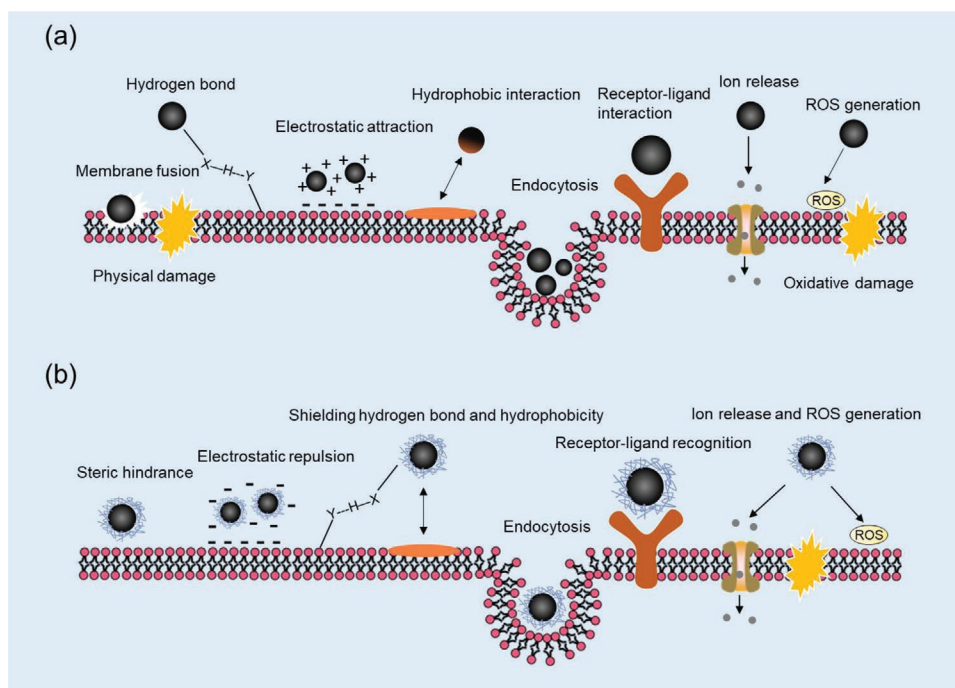


Figure 6. Altered interactions between ENMs and cell membrane by the acquisition of an environmental corona. a) The pristine ENMs can be adsorbed onto the cell membrane surface by hydrogen bonding, electrostatic attraction, hydrophobic interaction, receptor-ligand interaction, etc., causing internalization and membrane physical damage. ENMs also release ions and enter cells through ion channels, producing toxicity. In addition, some ENMs also produce extracellular ROS, causing membrane oxidative damage allowing ENM entry. b) The acquisition of an environmental corona can affect the adhesion of ENMs to the surface of cell membrane by increasing steric hindrance, electrostatic repulsion as most coronas are found to be slightly negatively charged, and shielding effects. In addition, it can also affect ion release and ROS generation, thereby reducing membrane damage. If there are specific biomolecules in the corona that engage with the cellular receptors enhanced uptake can be facilitated.

trigger cellular impairments through diverse molecular mechanisms (**Figure 6**). Endocytosis is the main transmembrane pathway by which cells internalize ENMs from the plasma membrane and deliver them to acidic endosomes or lysosomes for degradation, or recycle them back to the cell surface.^[194] In addition, phagocytosis and macropinocytosis are two other pathways involved in the cellular uptake of ENMs.^[195] CuO NPs have been found to traverse across the pore of the cell wall of *M. aeruginosa*, internalize across the plasma membrane, and reduce to Cu₂O in the intracellular environment.^[196] Comparatively, the internalization of CuO NPs by the algae was enhanced by Suwannee River FA. A further study reported that CuO NPs adhered to the surface of algal cells, interacted with their excreted EPS, and could be internalized via endocytosis and stored in algal vacuoles.^[141] These findings imply that cellular endocytosis of Suwannee River FA or EPS-coated CuO NPs occurred in algal cells. Recently, mixtures of BSA and HA have been utilized to simulate NOM for their investigation into the cellular uptake and intracellular trafficking of Ag NPs in *Tetrahymena thermophila* (*T. thermophila*).^[197] Interestingly, it was found that the BSA-coating enhanced the uptake rate of Ag NPs in *T. thermophila* via the caveolae-mediated pathway, while HA had no effect, and BSA-coating induced the transfer of Ag NPs into a more acidic intracellular environment. In contrast, another study reported that the lysosome was the target of Ag NPs in rainbow trout gut cells, whilst the protein corona was protective against cellular accumulation and cytotoxicity of Ag

NPs.^[198] Through the identification of hard protein coronas of NH₂- and COOH-PS NPs formed in the celomic fluid of the *Paracentrotus lividus*,^[199] some vital proteins involved in cell association and internalization in the corona of both NP types have been realized. This includes twosome and flotillin-1, which are likely to promote the cellular internalization of PS NPs.

4.4. Oxidative Stress

Oxidative damage is one of the major toxicological consequences of ENMs.^[177] Normally, extracellular and intracellular redox homeostasis is balanced by free radical production and antioxidant defense. ENMs are able to disrupt redox homeostasis and cause the excessive accumulation of ROS, such as superoxide anions ($\bullet\text{O}_2^-$), hydroxyl radicals ($\bullet\text{OH}$), and hydrogen peroxide (H_2O_2). These destroy proteins, DNA, and plasma membranes. Finally, ENMs may cause cell death if excess ROS cannot be cleaned up in time by the antioxidative system. Some ENMs, such as TiO₂ NPs, can generate ROS through a rapid reaction with oxygen, light-mediated photochemical transformations, or other reactions.^[200] It has been reported that NOM is able to influence the photochemical reactions of ENMs through direct surface binding or indirectly generating highly reactive intermediates as a photosensitizer or a ROS quencher.^[201,202] To address this issue, the effects of HA and FA on the ROS generation of sulfide NPs including WS₂, MoS₂, and CdS NPs

under ultraviolet irradiation have been compared.^[201] Results show that HA and FA decreased $\bullet\text{O}_2^-$, $\bullet\text{OH}$, and $^1\text{O}_2$ concentrations generated by WS_2 and MoS_2 NPs while promoting the $\bullet\text{O}_2^-$ generated by the CdS NPs. As a result, HA and FA exhibited limited alteration of the toxicity of CdS NPs toward *E. coli*, whilst decreasing the toxicity of WS_2 and MoS_2 NPs. Another study demonstrated that surface-bound HA on TiO_2 NPs may alleviate their toxicity to *Chlorella* sp. by depressing the generation of intracellular ROS.^[136] NOM in the Suwannee River also exhibited ROS quenching effects on TiO_2 NPs in an aquatic system, likely due to the redox properties of hydroquinone and phenol moieties in NOM substances.^[203] However, the effect of NOM on the phototoxicity of TiO_2 NPs was inconsistent between *D. magna* and *D. rerio* larvae, suggesting the presence of more complex toxicity processes, as opposed to ROS generation.^[203] The Suwannee River FA has been reported to enhance ROS generation, membrane, and cause DNA damage to *M. aeruginosa* from CuO NPs, by promoting the dissolution of CuO NPs and increasing the amount of small-sized CuO NPs and Cu^{2+} .^[196] Additionally, HA may elevate the ROS generated from Pd@Ir NPs, resulting in significantly enhanced bactericidal activity of the Pd@Ir NPs.^[125] Soluble EPS has also been found to alleviate TiO_2 NP-induced oxidative stress in *C. pyrenoidosa*.^[81] BSA decreased the Ag NP-induced ROS level in the white-rot fungus, *Phanerochaete chrysosporium*.^[81,204] However, it was reported that the protein corona formed from HS may increase ROS production induced by cationic NH_2 -PS NPs in *Mytilus* hemocytes, apparently mediated by the dysregulation of p38 mitogen-activated protein kinase signaling.^[160]

4.5. Immunotoxicity

In mammals, a large number of studies have demonstrated that ENMs have inherent immunotoxicity as exogenous particulate matter, via the activation of inflammation reaction, cytokine production, and related gene expression and signaling pathways.^[205] The immune systems of different aquatic organisms differ greatly between species. For example, gut epithelium and hepatopancreas are likely to be targeted by pollutants to cause immunotoxicity in crustaceans.^[206] In fish, many immune endpoints may be induced by pollutant-induced immunotoxicity, such as blood cells and immunological biomarkers.^[207] To date, there have only been few studies on the immunotoxicity of ENMs on aquatic organisms and even fewer studies on the role of acquired environmental corona. For instance, exposure to ZnO NPs increases the expression of complement component C4-2 in *Cyprinus carpio* L., an essential part of the innate immune system, involved in phagocytosis and inflammatory reactions.^[102] It was also reported that Ag NPs could affect the immunological parameters in the pronephros and liver of *Oncorhynchus mykiss*.^[208] Although protein corona can significantly affect the inflammatory response in mammals,^[88] the importance of environmental corona on the immunotoxicity of ENMs in aquatic organisms has rarely been reported. It is only known that, in body fluids of aquatic organisms, the protein corona on ENMs contains critical immunity molecules involved in immune system activation. In the HS of *M. galloprovincialis*, MgC1q6 has been identified as the only component of the hard

protein corona on NH_2 -PS NPs.^[160] In the celomic fluid of sea urchins, the twosome precursor protein has been identified as a dominant component in protein corona formed on NH_2 -PS NPs, triggering the interaction between the NH_2 -PS NPs and the sea urchin immune system cells (i.e., coelomocytes).^[209] Likewise, protein corona on TiO_2 NPs formed in the celomic fluid of sea urchins have been found to contain many proteins involved in cellular adhesion, cytoskeletal organization, and immune response.^[210]

4.6. Genotoxicity and Reproductive Toxicity

As discussed in Section 4.4, ENM exposure to aquatic organisms can lead to excessive intracellular ROS, producing genotoxic, and mutagenic effects. Additionally, it is possible that ENMs can accumulate inside the nucleus and directly interact with DNA or other genetic material as well as the secondary genotoxicity generated from ENM-induced responses.^[211] Genetic damage will lead to genomic mutation, abnormal cell cycle, and cell death. The detailed mechanisms underpinning these outcomes have been discussed in a recent paper, which reviewed the genotoxicity of metallic ENMs in aquatic organisms.^[211] However, most reported studies continue to focus on pristine ENMs, and overlook the significance of the environmental corona formed on ENMs in natural waters or during interaction with organisms. Thus, there continues to be uncertainty as to the impact of environmental corona on the genotoxic risks from ENMs, making it challenging to formulate conclusions on this relationship at present.

Beyond direct genetic damage, there may be possible adverse effects in subsequent generations of aquatic organisms. Toxicological studies have demonstrated the reproductive risks from ENMs in fish and sea urchins, inducing damage to ovarian and testicular tissues, oocytes, and sperms.^[212–215] However, these studies did not consider the altered reproductive effects from an environmental corona on ENMs, nor investigate changes in the offspring from ENM-treated parents. A recent study reported that protein corona on Ag NPs formed in the plasma of *Micropterus dolomieu* varied with fish sex, where the corona in male plasma was slightly thinner and had a less negative ζ -potential than the female plasma.^[103] For observed gender-specific corona, they demonstrated that the egg-specific proteins (i.e., vitellogenin and zona pellucida) were only identified in the corona derived from female plasma, indicating that the corona may affect the Ag NP-related reproductive toxicity and their accumulation in oocytes. A recent *D. magna* multigenerational study showed that medium-aged Ag and TiO_2 NPs in NOM-containing high-hardness synthetic water, had fewer overall toxicological consequences on growth and longevity across all generations in continuous and parent-only exposure scenarios than in a salt-only medium. This implies that the application of standard *D. magna* tests overestimate ENM toxicity with a salt-only medium.^[216] Furthermore, after aging for six months in environmentally realistic water containing NOM, there was a considerable reduction in the inhibitory effects on reproduction and antioxidant stress-related genes on *D. magna* over four generations from pristine Ag NPs.^[217]

5. Conclusions and Perspectives

Unlike traditional pollutants (e.g., persistent organic pollutants and heavy metals), ENMs as emerging pollutants, drive more complex environmental and biological interactions because of their extremely small particle size and high active surface. Both of these traits contribute to the dynamicity of ENMs. Once released into aquatic ecosystems, ENMs encounter diverse ecological macromolecules, and acquire an environmental corona at the interface of ENMs, generating a new eco-identification. The environmental corona is able to mediate the fate and ecotoxicity of ENMs in water systems. In particular, the inherent biological effects and interactions of pristine ENMs on aquatic organisms are significantly altered, a key feature of ENMs that has, to date, been neglected by most research.

To address this important issue, we systemically reviewed the current understanding of representative types of macromolecules adsorbed onto ENMs in aquatic ecosystems, which constitute the environmental corona; these include NOM, EPS, proteins, and surfactants. Their characteristics in the environmental corona are still unclear, lacking sufficient chemical and structural determination to understand their orientation and activity, particularly outside of the controlled laboratory environment or theoretical determinations. Next, using the literature, the ecotoxicological effects of environmental corona-coated ENMs on representative aquatic organisms were compared to those of pristine ENMs. In most cases, NOM and EPS coronas were reported to alleviate the bioavailability and hazards of ENMs to aquatic organisms because they stabilize or destabilize ENMs in aqueous medium. Protein coronas may aggravate the toxic risks of ENMs due to the diverse molecular functions of proteins in secretions or body fluids with which the corona-associated proteins can interact. Surfactant coronas may potentially enhance ENM toxicity due to the inherent toxicity of many surfactants themselves. Eventually, environmental corona-coated ENMs may trigger differential toxicological mechanisms in comparison with uncoated ENMs, such as varied cell membrane interactions and ROS generation.

Although some primary knowledge has been acquired via mimetic studies in the laboratory, the current understanding regarding the vital role of environmental coronas on ENMs in aquatic ecosystems remains insufficient. Specific problems and challenges need to be fully appreciated to enable a better appraisal of the ecological and health risks from ENMs in aquatic ecosystems and organisms, as summarized below:

i) Many studies have investigated the properties of the environmental corona formed in mimetic aqueous media and have provided many important clues demonstrating the importance of environmental corona on ENMs. These studies are generally set in the laboratory with commercial chemicals, such as NOM, EPS, and proteins. However, the complicated composition of macromolecules in natural water bodies, including their seasonal variability, is not well simulated within a laboratory setting, as the corona must dynamically change with the surrounding medium. To date, there is a lack of sufficient experimental evidence and information on detailed chemical composition of environmental corona on ENMs in aqueous media as well as the adsorption

energetics and adsorption modes.^[31,218] As such, in situ characterization of corona formation and evolution on the surface of ENMs in natural waters is required. This should be coupled with more integrated analytical techniques and convincing methodologies, such as FT-ICR-MS, FT infrared spectroscopy, Raman spectroscopy, and capillary electrophoresis mass spectrometry; these are powerful tools that may elucidate the structural-activity relationships that aid in predicting the potential ecotoxicological risks of ENMs;

ii) Even if the extrinsic aquatic factors remain consistent, the intrinsic physicochemical properties of multifarious ENMs (e.g., metallic, metal oxide, carbonaceous, and polymeric NPs) may produce very unique characteristics in their acquired coronas. Typically, hydrophobic and hydrophilic ENMs exhibit distinct abilities to adsorb ecological macromolecules. For analogous ENMs such as anatase and rutile TiO₂, their varying surface properties (e.g., lattice plane, charge, and ligands) can impact corona formation.^[219] Pristine ENMs may also undergo various environmental transformations (e.g., oxidation, sulfidation, reduction, and dissolution), generating a greater number of unpredictable changes to ENMs and their resulting environmental coronas.^[38] To acquire in-depth analysis and solid conclusions, a greater number of ENMs should be tested for comprehensive assessment and comparison, rather than limited types. More importantly, the establishment of such ENM libraries and a wide spectrum of NOM and EPS standards, will facilitate efforts to uncover the underlying mechanisms of corona formation and their related impacts on ecotoxicological studies.^[180] High-throughput approaches may also be applied to predict the formation of coronas and their structural features, such as statistical and machine learning strategies, which may advance the cognition of environmental coronas on ENMs with less time and labor-consuming processes;^[180,220]

iii) Based on the literature, there are inconsistent and conflicting findings regarding the impacts of environmental coronas on ENM ecotoxicity. For example, some studies have demonstrated that EPS may shield the toxic effects of ENMs on bacteria or algae, whilst enhanced toxicity was also observed.^[141] Thus, in addition to the aqueous conditions and ENM types, the application of more representative aquatic species, such as bacteria, algae, rotifers, copepods, shellfish, and fish is required in conjunction with a mixture of their secreted biomolecules. A battery of aquatic biota may be used for toxicological assessment of corona-coated ENMs, representing diverse key functions at the ecosystem level. Aquatic organisms at lower and upper trophic levels may be used to study the food chain transfer and bio-magnification of corona-coated ENMs from single-celled organisms to higher organisms;^[221]

iv) When NOM or EPS-coated ENMs move from the exterior aquatic environment into an intracorporeal or intracellular environment, the macromolecules in the surrounding medium will change into proteins, lipids, and nucleic acids. This is due to the exchange and evolution of the corona. However, there is a lack of clarity as to whether the adsorbed NOM or EPS macromolecules will be covered or replaced by other biomolecules and form an evolutionary corona inside

cells or organisms. The consequences of displaced biomolecules as well as their interplay processes are also not clearly understood. In addition, the role of secreted proteins or other macromolecules in the environmental corona of ENMs on cellular internalization, intracellular fate, or transformation is still under investigation;

- v) Compared to toxicological studies on mammals, knowledge of differential biological effects and underlying mechanisms between pristine ENMs and corona-coated ENMs is relatively limited in aquatic organisms. The most altered interactions from the environmental corona appears to be membrane adhesion, membrane damage, cellular internalization, and oxidative stress responses induced by ENMs. In contrast, effects such as genotoxicity and reproductive toxicity, and the role of the environmental corona, are only beginning to be explored. In addition to specific classical toxic endpoints, such as DNA-strand breaks and apoptosis, a greater number of hazardous outcomes and mechanisms should be considered, such as cell differentiation, epigenetic modification, gene mutation, and enzyme inactivation. Moreover, new toxicological mechanisms of ENMs have recently been elucidated, such as ferroptosis,^[222] enzyme-mimicking activity,^[223] and extracellular multitarget invasion mechanism;^[224] these have not yet been identified in environmental corona-coated ENMs. To date, we are unaware of any studies performed to assess the biological effects of NOM or EPS-coated ENMs on mammals; this is considered critical in evaluating their potential risks to humans without under or overestimating risk;
- vi) In aquatic ecosystems, released ENMs coexist with many other pollutants (e.g., persistent organic pollutants, heavy metals, personal care chemicals, pharmaceuticals), and their coexistence may result in enhanced or weakened toxicity. Commonly, the presence of environmental coronas may affect the adsorption, desorption, and degradation of pollutants on ENMs, altering their synergistic or antagonistic effects on aquatic organisms. For instance, due to their high surface-to-volume ratio and numerous oxygen functional groups, carbonaceous nanomaterials (e.g., GO and CNTs) readily adsorb free heavy metal ions (e.g., Cd²⁺ and Cu²⁺) in aqueous media. This may significantly aggravate or weaken the toxicity of heavy metals.^[190,225] However, the potential roles of environmental corona on the adsorption and desorption properties of carbonaceous nanomaterials toward heavy metal ions are poorly understood, and an investigation into the underlying interfacial reactions is urgently required.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

aquatic environment, aquatic organisms, ecotoxicological effects, engineered nanomaterials, environmental corona

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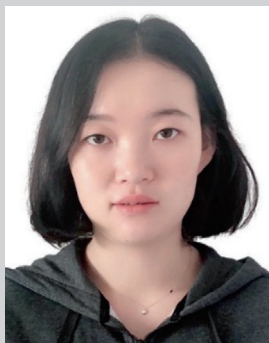
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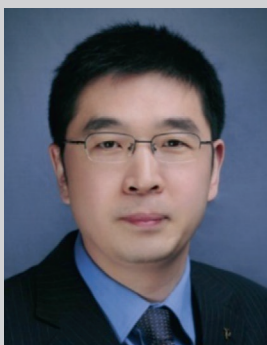
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